(19) Four scales are based upon solvent effects on electronic transitions, one on IR stretching vibration, one on ^{19}F NMR shifts, one on the nitrogen hyperfine splitting constant of nitroxides, one of the kinetics at 20 °C of a selected Menschutkin reaction, and one is a composite of spectroscopic, kinetic, and thermodynamic properties.
(20) Very similar conclusions have recently been reached by Kalyanasundaram and Thomas through the study of medium effects on vibronic intensities of monomeric pyrene fluorescence (K. Kalyanasundaram and J. K. Thomas, *J. Am. Chem. Soc.*, **99**, 2039 (1977).
(21) H. Block and S. M. Walker, *Chem. Phys. Lett.*, **19**, 363 (1973).
(22) L. Onsager, *J. Am. Chem. Soc.*, **58**, 1486 (1936).
(23) J. L. Abboud and R. W. Taft, unpublished work.
(24) On the same grounds, we consider that the use of solvents 6, 8, 9, 10, 12, 14, 15, 17, 20, 21, 22, 24, 26, 30, 31, 33, 35, 36, 37, 43, 44, 46, 47, 49, 53, 57, 101–113, 201, and 202 of ref 2 is not suitable for these purposes.
(25) (a) Naval Surface Weapons Center; (b) Visiting Scientist, UCI, 1976–1977.

José Luis Abboud, Mortimer J. Kamlet,[25] R. W. Taft*
Department of Chemistry, University of California
Irvine, California 92717
Received September 22, 1977

Application of Deuterium Magnetic Resonance to Biosynthetic Studies. 2. Rosenonolactone Biosynthesis and Stereochemistry of a Biological S_N2' Reaction

Sir:

The introduction of ^2H NMR as a biosynthetic technique[1-4] offers a powerful tool for the examination of subtle stereochemical questions heretofore accessible only with the use of tritiated substances and lengthy degradation procedures. To demonstrate the utility of this new method, potential problems of sensitivity and resolution must be overcome. In exploring the limits of ^2H NMR we have examined the stereochemistry of the formation of ring C in the biosynthesis of the fungal diterpene rosenonolactone (**1**).

The classic studies of Arigoni[5] and Birch[6] and the subsequent work of Hanson[7] have established many of the details of the biosynthesis of rosenonolactone. According to the accepted Scheme I, electrophilic cyclization of geranylgeranyl pyrophosphate, formed from four molecules of mevalonate, gives the bicyclic labda-8(17),13-dien-15-yl pyrophosphate (**2**). A second cyclization involving allylic displacement of the terminal pyrophosphate and concomitant hydride and methyl migrations generates ring C. The exact timing of lactone formation is as yet unknown.

The allylic displacement by which ring C is formed may formally be considered an S_N2' process. To determine the stereochemistry of this process one must answer two questions. (1) Which face of the 13,14 double bond of **2** is attacked by the terminal methylene? From the known absolute configuration of **1**,[8] it follows that cyclization occurs on the *si* face of the allyl system. (2) In which sense, syn or anti, does the pyrophosphate depart? This question may be answered by observing which

Scheme I

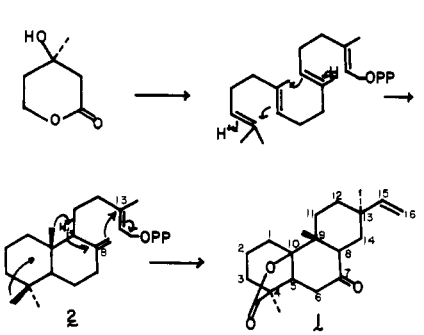
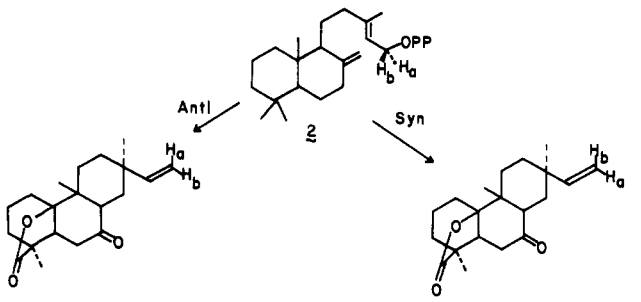


Table I. Incorporation of [5-^2H]Mevalonates into **1**

Mevalonate (mmol)	**1**, mg	Incorpn, %[a]	Enrichment, %
[5-$^2H_2$] (8.1)	70	1.1	5.2
(5*R*)- (4.1)	30	0.55	3.0
(5*S*)- (4.6)	30	1.1	5.7

[a] Based on (3*R*)-mevalonate.

Scheme II



of the prochiral hydrogens at C-16 of **2** becomes cis (H-16*Z*) and which becomes trans (H-16*E*) to the C–C bond in the terminal vinyl group of rosenonolactone (Scheme II). We describe below the solution of this problem using ^2H NMR.

Analysis of the 270-MHz ^1H NMR of rosenonolactone ($CDCl_3$) allows, inter alia, the following proton assignments. (1) The vinyl group appears as an ABX pattern, J_{AB} = 1.0 Hz, J_{AX} = 17.5 Hz, J_{BX} = 10.6 Hz. The individual protons were assigned on the basis of known cis and trans coupling constants for olefinic hydrogens:[9] H-16*Z* = 4.97 ppm, H-16*E* = 4.90 ppm, H-15 = 5.80 ppm. (2) The methylene protons adjacent to the ketone may also be assigned from analysis of coupling constants: H-6β = 2.12 ppm, H-6α = 2.39 ppm ($J_{H\text{-}6\alpha\text{-}H\text{-}6\beta}$ = 13.7 Hz, $J_{H\text{-}5\text{-}H\text{-}6\beta}$ = 16.5 Hz, $J_{H\text{-}5\text{-}H\text{-}6\alpha}$ = 3.7 Hz).

A series of specifically deuterated substrates, sodium [5-$^2H_2$]mevalonate,[10] (5*R*)-[5-^2H]mevalonate,[11] and (5*S*)-[5-^2H]mevalonate,[11] each mixed with [2-^{14}C]mevalonate to allow calculation of enrichments, was fed to four-day-old cultures of *Trichothecium roseum* (ATCC 8685).[16] After an additional 7 days the mycelia were harvested by filtration, dried, powdered, and extracted with hexane for 24 h. The concentrate was triturated with pentane and the residue recrystallized seven–ten times from methanol to give rosenonolactone which was free of persistent traces of isorosenonolactone and small quantities of highly deuterated impurities. These experiments are summarized in Table I.

Each of the biosynthetically deuterated samples was analyzed by ^2H NMR.[17] Rosenonolactone (**1A**), derived from feeding of [5-$^2H_2$]mevalonate, shows a signal at 4.97 ppm with a shoulder at ~4.90 ppm. A sample of authentic [16-$^2H_2$]-rosenonolactone (**1D**)[19] gives an identical spectrum in the olefinic region as does a mixture of *cis*- and *trans*-[16-^2H]-rosenonolactone (**1E**).[22] The terminal methylene signals, separated by only 0.07 ppm (3 Hz), are therefore not clearly resolved. From the peak shape it was inferred that the observed signal results from the superposition of two resonances of unequal line width, the higher field signal being the broader. This conclusion was confirmed in the sequel (see below). The spectrum of **1A** also has the expected signals at 2.34 and 2.08 ppm corresponding to H-6α and H-6β, respectively. The remaining signals at 1.89, 1.71, and 1.42 ppm are presumably due to deuterium at C-2 and C-11. Rosenonolactone (**1B**), derived from (5*R*)-[$^2H_1$]mevalonate exhibits a signal at 5.01 ppm ($\nu_{1/2}$ = 3.5 Hz) while **1C** (from (5*S*)-[$^2H_1$]mevalonate) gives rise to a signal at 4.92 ppm ($\nu_{1/2}$ = 7.5 Hz). The positions of the observed signals were confirmed by doping each sample with ~1/3 part of **1E**: the signal from **1B** plus **1E** shows an up-

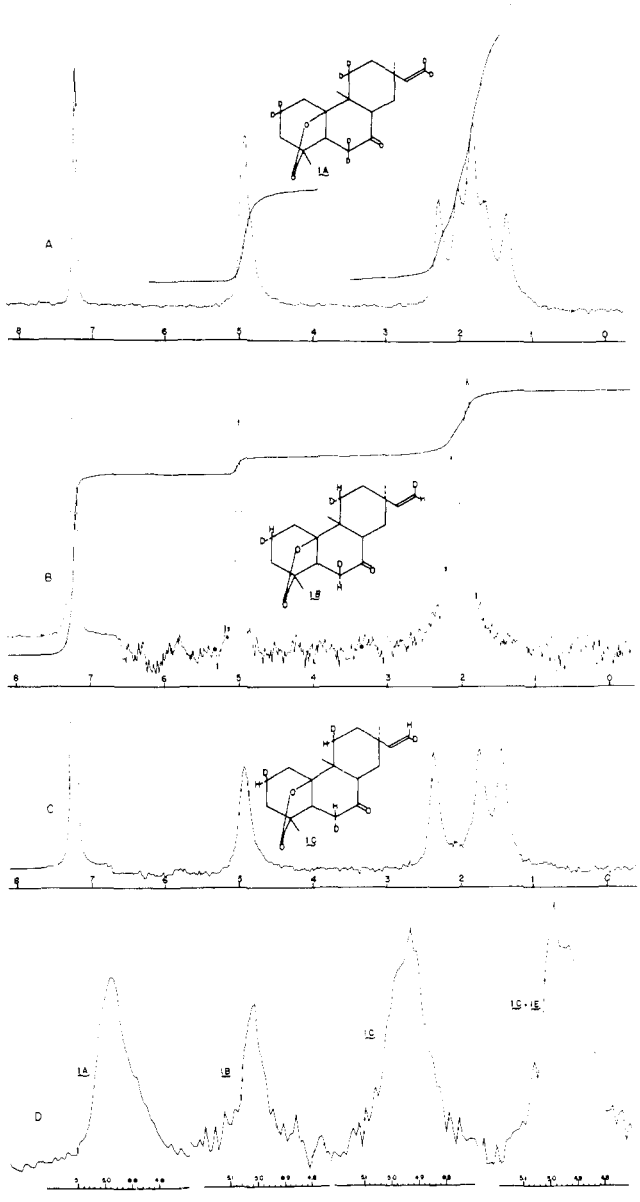


Figure 1. Proton decoupled ^2H NMR spectra of labeled **1:** A, 0.13 mmol of **1A** (from feeding of [5-$^2H_2$]mevalonate), 7030 transients, line broadening (LB) = 0.5 Hz; B, 0.032 mmol of **1B** (from feeding of (5*R*)-[^{2}H]-mevalonate), 27970 transients, LB = 0.5 Hz; C, 0.085 mmol of **1C** (from feeding of (5*S*)-[^{2}H]mevalonate), 6708 transients, LB = 0.5 Hz; D, expanded spectra of olefinic regions of deuterated rosenonolactone samples, including mixture of **1C** and ~30% **1E.**

field tail, while that from **1C** plus **1E** has maxima at 4.98 and 4.93 (cf. Figure 1D).

In accord with previous stereochemical studies of isoprenoid biosynthesis,[23] the signal corresponding to H-6β (2.13 ppm) is enhanced in the spectrum of **1B.** Similarly a signal due to H-6α (2.37) appears in the spectrum of **1C** (Figure 1).

The above observations establish that the 5-pro-*R* hydrogen of mevalonate becomes the 16*Z* hydrogen of rosenonolactone. Conversely the 16*E* hydrogen of **1** is derived from the 5-pro-*S* hydrogen of mevalonate. These results, taken together with the known direction of attack on the 13,14 double bond of **2,** establish that the allylic displacement which generates ring C of **1** takes place with overall anti (or antarafacial) stereochemistry.[24]

The S_N2' reaction has been the subject of considerable interest as well as substantial controversy. While there are few unambiguous examples of true S_N2' processes,[25] Stork[26a] has reconfirmed his original report of syn stereochemistry in the reaction of a 2-cyclohexenyl-1-dichlorobenzoate with piperidine. On the other hand use of an acyclic substrate and sulfide nucleophile resulted in a significant anti component to the reaction.[26b] Arigoni has recently demonstrated anti stereochemistry in the solvolysis of linalool to α-terpineol.[27] Also noteworthy is the work of the Roussel group in which an S_N2' reaction of an allylic epoxide was used to generate a prostaglandin with the requisite stereochemistry.[28] Theoretical calculations supporting both syn and anti pathways are available.[29] The above ^2H NMR study is the first explicit determination of the stereochemistry of a biochemical "S_N2'" reaction. It remains to be seen whether the observed stereochemical restraints also apply to other enzymatically controlled nucleophilic allylic displacement processes.[30]

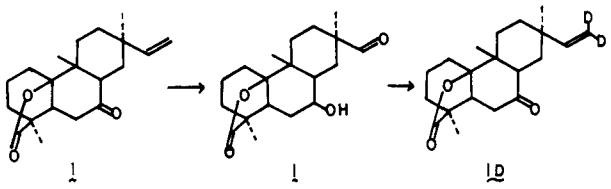
References and Notes

(1) Y. Sato, T. Oda, and H. Saito, *Tetrahedron Lett.*, 2695 (1976); Y. Sato, T. Oda, and H. Saito, *J. Chem. Soc., Chem. Commun.*, 415 (1977).
(2) B. W. Bycroft, C. M. Wells, K. Corbett, and D. A. Lowe, *J. Chem. Soc., Chem. Commun.*, 123 (1975).
(3) P. M. Dewick and D. Ward, *J. Chem. Soc., Chem. Commun.*, 338 (1977).
(4) D. E. Cane and S. L. Buchwald, *J. Am. Chem. Soc.*, **99,** 6132 (1977).
(5) D. Arigoni, *Ciba Found. Symp. Biosynth. Terpenes Sterols, 1958,* 239–242 (1959); J. J. Britt, *Diss. ETH (Zurich),* No. 2948 (1959); J. J. Britt and D. Arigoni, *Proc. Chem. Soc.*, 224 (1958).
(6) A. J. Birch and H. Smith, *Ciba Found. Symp. Biosynth. Terpenes Sterols, 1958,* 259–260 (1959); A. J. Birch, R. W. Richards, H. Smith, A. Harris, and W. B. Whalley, *Tetrahedron,* **7,** 241 (1959).
(7) B. Achilladelis and J. R. Hanson, *Phytochemistry,* **7,** 589 (1968); B. Achilladelis and J. R. Hanson, *Tetrahedron Lett.*, 4397 (1968); B. Achilladelis and J. R. Hanson, *Chem. Commun.*, 488 (1969); B. Dockerill and J. R. Hanson, *J. Chem. Soc., Perkin Trans. 1,* 324 (1977).
(8) W. B. Whalley, B. Green, D. Arigoni, J. J. Britt, and C. Djerassi, *J. Am. Chem. Soc.*, **81,** 5520 (1959); C. Djerassi, B. Green, W. B. Whalley, and G. G. DeGrazia, *J. Chem. Soc. C,* 624 (1966); A. I. Scott, S. A. Sutherland, D. W. Young, L. Guglielmetti, D. Arigoni, and G. A. Sim, *Proc. Chem. Soc.*, 19 (1964).
(9) L. M. Jackman and S. Sternhell, Ed., "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Second ed, Pergamon Press, Oxford, 1969, p 278.
(10) Synthesized as previously described: ref 4 and J. W. Cornforth and R. H. Cornforth, *Biochem. Soc. Symp.,* **No. 29,** 5 (1969).
(11) (5*R*)-[5-^{2}H]Mevalonolactone and (5*S*)-[5-^{2}H]mevalonolactone were synthesized from (1*R*)-[1-^{2}H]- and (1*S*)-[1-^{2}H]-3-methyl-3-buten-1-ol by successive reaction with *N*-bromosuccinamide in water, potassium cyanide, and sodium hydroxide according to Cornforth.[12] The requisite isopentyl alcohols were in turn prepared by the diaphorase exchange method of Simon[13] and the stereochemical assignments verified by Gerlach's camphanate ester procedure.[14] Thus in a typical reaction 1.5 g (17.4 mmol) of isopentyl alcohol in 25 mL of pH 6.8 (pD 7.2) 0.1 M phosphate buffer (D_2O) was added slowly under nitrogen to a mixture of 125 mg (0.19 mmol) of NAD^+, 60 mg of horse liver alcohol dehydrogenase (Boehringer-Mannheim, 2.8 U/mg), 20 mg (2.0 ml) of diaphorase (Boehringer-Mannheim, 210 U/mg), and 200 mg of bovine serum albumin in 175 mL of the same buffer (D_2O). After 24 h at room temperature an additional 10 mg of diaphorase and 20 mg of dehydrogenase were added and incubation continued for a total of 55 h. The reaction was stopped by saturation with sodium chloride and the labeled isopentenol recovered by continuous extraction with ether (55–60% yield). A lanthanide shift analysis (Eu(DPM)$_3$) of the derived camphanate ester showed that only the 1-pro-*R* hydrogen had been exchanged (80–90%). A small amount of nonstereospecific exchange occurred at C-2 (15%) (mass spectrum: 13.8% d_0, 70.4% d_1, 15.8% d_2). The corresponding (1*S*)-[1-^{1}H]isopentyl alcohol was generated by inversion of (1*R*)-[1-^{1}H]isopentyl alcohol via the benzoate ester and subsequent lithium aluminum hydride reduction.[15] Analysis of the camphanate ester, as above, verified the stereochemical homogeneity of the (1*S*)-[1-^{1}H]isopentyl alcohol.[14] Experimental details will be given in the full paper.
(12) J. W. Cornforth, F. P. Ross, and C. Wakselman, *J. Chem. Soc., Perkin Trans. 1,* 429 (1974); J. W. Cornforth and F. P. Ross, *Chem. Commun.*, 1395 (1970).
(13) H. Guenther, F. Biller, M. Kellner, and H. Simon, *Angew. Chem., Int. Ed. Engl.,* **12,** 146 (1973).
(14) H. Gerlach and B. Zagalak, *J. Chem. Soc., Chem. Commun.*, 274 (1973).
(15) A. K. Bose, B. Lal, W. A. Hoffman, and M. S. Manhas, *Tetrahedron Lett.*, 1619 (1973); O. Mitsunobu and M. Eguchi, *Bull. Chem. Soc. Jpn.,* **44,** 3427 (1971).
(16) An initial incorporation of [5-$^3H_2$,2-^{14}C]mevalonate ($^3H/^{14}C$, 3.15) gave rosenonolactone ($^3H/^{14}C$, 2.98) reconfirming the essentially complete retention of label from C-5 of mevalonate.
(17) Proton decoupled ^2H NMR spectra were obtained at 41.44 MHz on a Bruker HX 270 operated in the FT mode. Sample tubes (10 mm) equipped with 475-μL cylindrical inserts and containing degassed $CHCl_3$ solutions with $CDCl_3$ as internal standard (δ 7.24, $\nu_{1/2}$ 1.5–2.0 Hz) were used. A pulse angle of 90° was employed, spectral width 500 Hz, 2K data points. Chemical shifts are ±0.02 ppm. Although it has been pointed out that proton and deuterium chemical shifts are interchangeable,[18] this is of course true only under *identical* conditions. In practice small variations in peak position

are observed from one sample to another. Critical assignments (vide supra) must be made with the use of added internal standards or other methods.

(18) P. Diehl in "Nuclear Magnetic Resonance Spectroscopy of Nuclei Other than Protons", T. Axenrod and G. A. Webb, Ed., Wiley, New York, N.Y., 1974.

(19) Synthesized by reduction of **1** with sodium borohydride to rosenololactone followed by oxidation to the noraldehyde i using osmium tetroxide–sodium periodate,[20] Wittig reaction with the ylide generated from methyl-[$^2H_3$]-triphenylphosphonium iodide,[21] and Jones oxidation.



(20) R. Pappo, D. S. Allen, R. U. Lemieux, and W. S. Johnson, *J. Org. Chem.*, **21**, 478 (1956).

(21) D. Seyferth, W. B. Hughes, and J. K. Heeren, *J. Am. Chem. Soc.*, **87**, 2847 (1965).

(22) Prepared by reaction of the noraldehyde i with the Wittig reagent formed by treatment of methylene triphenylphosphorane with 1 equiv of perdeuterioacetic acid and remetalation with *n*-butyllithium, followed by Jones oxidation of the resulting rosenololactone: **1E**, 35% d_0, 42% d_1, 23% d_2.

(23) J. W. Cornforth, *Chem. Soc. Rev.*, **2**, 1 (1973).

(24) Because the signals for H-16*E* and H-16*Z* are poorly resolved, as much as 20% syn component would not have been detected. For the moment we are assuming complete stereospecificity for the enzyme-catalyzed process. This point is currently under investigation.

(25) Cf. F. G. Bordwell, *Acc. Chem. Res.*, **3**, 281 (1970).

(26) (a) G. Stork and A. Kreft, *J. Am. Chem. Soc.*, **99**, 3850 (1977); G. Stork and W. N. White, *ibid.*, **75**, 4119 (1953); **78**, 4609 (1956). (b) G. Stork and A. F. Kreft, *ibid.*, **99**, 3851 (1977).

(27) S. Gotfredsen, J. P. Obrecht, and D. Arigoni, *Chimia*, **31** (2), 62 (1977). The authors have suggested a possible analogy between this S_Ni' cyclization and the biosynthesis of mono- and sesquiterpenes.

(28) J. Martel, E. Toromanoff, J. Mathieu, and G. Nomine, *Tetrahedron Lett.*, 1491 (1972).

(29) W. Drenth, *Recl. Trav. Chim. Pays-Bas*, **86**, 318 (1967); R. L. Yates, N. D. Epiotis, and F. Bernardi, *J. Am. Chem. Soc.*, **97**, 6615 (1975).

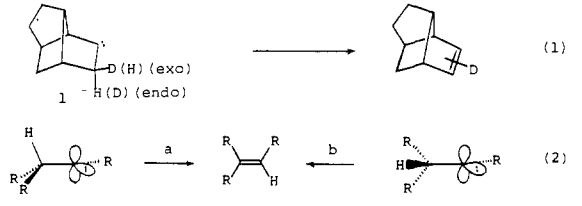
(30) This work was supported by the National Science Foundation (PCM 74-07924) and by a grant from the Eli Lilly Co. The Bruker HX 270 facility is supported by NIH Grant No. 1-P07-PR00798 from the Division of Research Resources.

David E. Cane,* Pushpalatha P. N. Murthy
Department of Chemistry, Brown University
Providence, Rhode Island 02912
Received September 12, 1977

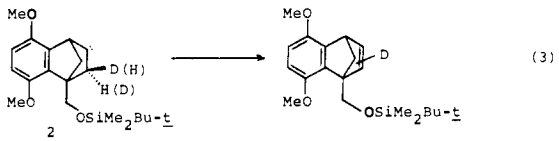
4-*tert*-Butyl-2,2-dimethylcyclohexylidene. A Surprising Lack of Stereoselectivity in a 1,2-Hydrogen Shift to an Alkylcarbene

Sir:

Recently it was reported that the exo/endo-H migratory ratio in brexan-5-ylidene **1** was 138 (eq 1).[1] Examination of models indicates that **1** is sufficiently distorted so that the exo H is much closer to alignment with the empty p orbital on the carbene center than is the endo H. Thus the migratory ratio greatly favoring exo-H migration might be interpreted as an affirmation of a number of theoretical predictions which state that the hydrogen which migrates is that which aligns with the empty p orbital (eq 2, path a rather than b is favored).[2] Our

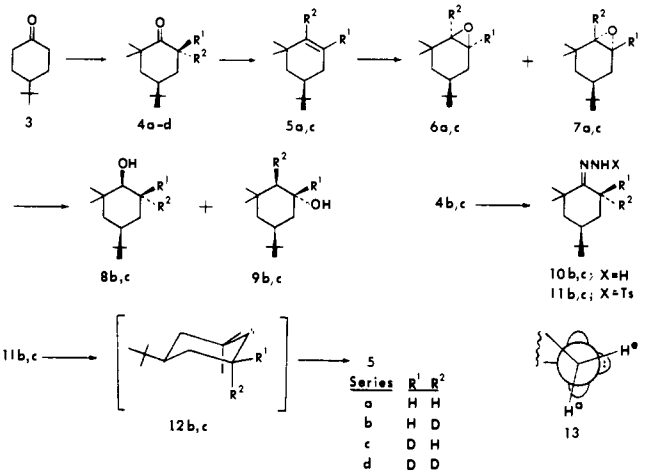


more recent work indicated, however, that cautious interpretations of the elegant experimental work of Nickon and his coworkers were in order, since, in an apparently unbiased[3] bicyclo[2.2.1]carbene **2** (eq 3), the exo-H/endo-H preference was 13 (at 190 °C).[4] Thus it might be that factors other than stereoelectronic control are operative in bicyclo[2.2.1]carbene systems.



To investigate stereoelectronic control of 1,2-H shifts in alkylcarbenes without the attendant ambiguities described above, we chose the substituted cyclohexylidene **12** as the reactive intermediate to be studied. Inspection of a Dreiding model of **12** indicates that this conformationally rigid carbene[5] has the axial hydrogen atom (H^a) ~10° away from alignment with the empty orbital and the equatorial hydrogen (H^e) is ~10° away from alignment with the sp^2 orbital as shown by the Newman projection **13.** Thus this system appeared to be an excellent choice to probe the question of stereoelectronic control of 1,2-H shifts in alkylcarbenes.

The synthesis of the carbene precursors **11** began with 4-*tert*-butylcyclohexanone (**3**), which was converted in 40%



overall yield to ketone **4a**[6] via Coates' procedure[7] of reduction-alkylation of the *n*-butylthiomethylene derivative of **3**.[8] Olefin **5a**[9] was obtained in 65% overall yield from **4a** by the thermolysis (525 °C) of a pentane solution of the acetates of the alcohols derived from the $LiAlH_4$ reduction of **4a.** Epoxidation of **5a** with MCPBA in chloroform solution gave a 1:4 mixture of cis and trans epoxides **6a** and **7a** (85%), which was reduced with $LiAlD_4/AlCl_3$ (2.5/1 mol ratio)[10] in ether to give alcohols **8b** and **9b** in quantitative yield. Alcohol **8b**[6] was obtained pure (41% from the epoxides) by careful fractional crystallizations from hexane of the *p*-nitrobenzoates of **8b** and **9b,** followed by hydrolysis (KOH/MeOH). Brown oxidation[11] of **8b** gave **4b** (83%), with $d_0:d_1:d_2$ = 2:98:0.[12a] Ketone **4a** was converted to **4d** ($d_0:d_1:d_2$ = 1:5:94) by a series of exchange reactions using $DO^-/D_2O/THF$, and **4d** was then transformed into **4c** ($d_0:d_1:d_2$ = 5:95:0)[12b] through the above-described series of reactions, except that the epoxides **6c** and **7c** were opened by $LiAlH_4/AlCl_3$.

Our attempts to generate **11b** and **11c** by reaction of ketones **4b** and **4c** with *p*-toluenesulfonyl hydrazide were thwarted because all such reactions, under a variety of conditions, led to extensive loss of deuterium.[13] We discovered, however, that reaction of **4b** and **4c** with distilled, anhydrous hydrazine[14] in refluxing methanol for 18 h gave hydrazones **10b** and **10c** (70%) with no exchange. Treatment of **10b** with 1 equiv of *n*-BuLi in ether at −78 °C and then addition of the resulting solution to tosyl chloride (1.4 equiv) in THF at −78 °C gave a mixture containing two major components, one of which was